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Deep Subsurface Microbiology and the Homestake Gold Mine

Terry C. Hazen

Center for Environmental Microbiology

Lawrence Berkeley National Laboratory

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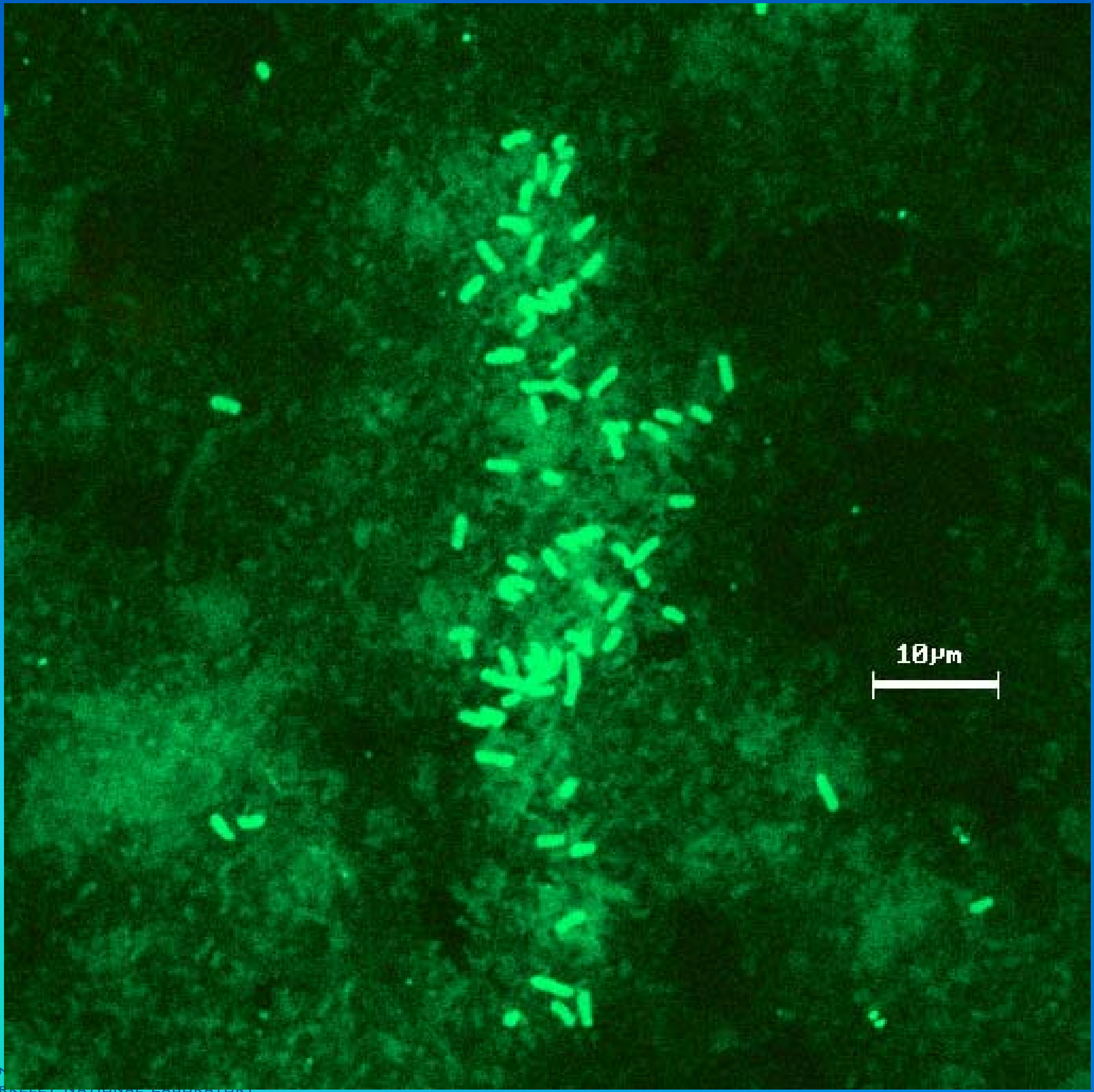

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Microbial Growth Capabilities

Factor	Lower Limit	Upper Limit
Temperature	-12˚C	104˚C at 1000 ATM (sulfate reducing & oxidizing bacteria)
Eh	-400 to -450 mv at pH8 (CH_4 producing bacteria)	+850 mv at pH 3 (from bacteria)
pH	0 to 0.5	>13 *Thiobacillus thioxidans* *Plectonema sp.*
Hydrostatic	0 Pressure	1400 ATM (deep sea bacteria)
Salinity	Double Distilled H_2O	Saturated Brines (halophilic bacteria)
Heavy Metals	<0.01 ppb	20,000 ppm Hg
Gases	CO_2, N_2, CH_4, H_2S, H_2	

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Microbial Life on Earth

■ Open Ocean	1.2×10^{29} cells
■ Soil	2.6×10^{29}
■ Oceanic Subsurface	3.5×10^{30}
■ Terrestrial Subsurface	$0.25\text{-}2.5 \times 10^{30}$
■ All sources	$4\text{-}6 \times 10^{30}$

- 350-550 Pg of Carbon (60-100% of plants & animals)
- 85-130 Pg of N and 9-14 Pg of P
- 10^5-10^7 species
- 4 simultaneous mutations in every gene in 0.4-170 hours

(Whitman et al., 1998)

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Do we know what's down there?

ISOLATE*	%G+C	%DNA hom	SITE
P. aeruginosa	67.0	100	
A01270	54.4	52.3	P28
C0198	54.7	50.9	P29
B0259	64.4	8.3	P24
A0111	66.2	0.0	P28
A0232	64.7	29.4	P28
A1271	46.8	45.1	P28
C0679	62.5	41.4	P29

*phenotypically all isolates were *P. aeruginosa*

Berkeley Lab

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Other Programs

- DOE (OBER) Subsurface Science Program 1985-98
- NSF Life in Extreme Environments 1997-present
- Russian Studies in Oil Fields 1950-1970
- NASA Astrobiology Program 1999-present

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Gold Mine Isolates

- Unique niches
  - Kieft et al. (1999) Thermus isolate that uses O_2, NO_{3-}, Fe(III), and S0 as terminal electron acceptors for growth isolated from 3.2-km in South Africa, optima 65°C
  - Santini et al. (2000) Chemolithoautotrophic Proteobacteria that utilizes arsenite as electron donor and O_2 as electron acceptor, Australian gold mine

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Oil Reservoir Isolates

- **Thermophilic and Halophilic Archaea**
  - Slobodkin et al. (1999) dissimilatory Fe (III) reducer from western Siberia.
  - Nazina et al. (2001) 14 Geobacillus isolates Russia, Kazakhstan and China
  - Orphan et al. (2000) Thermobacterium, Pseudomonas, etc. from California and Canada

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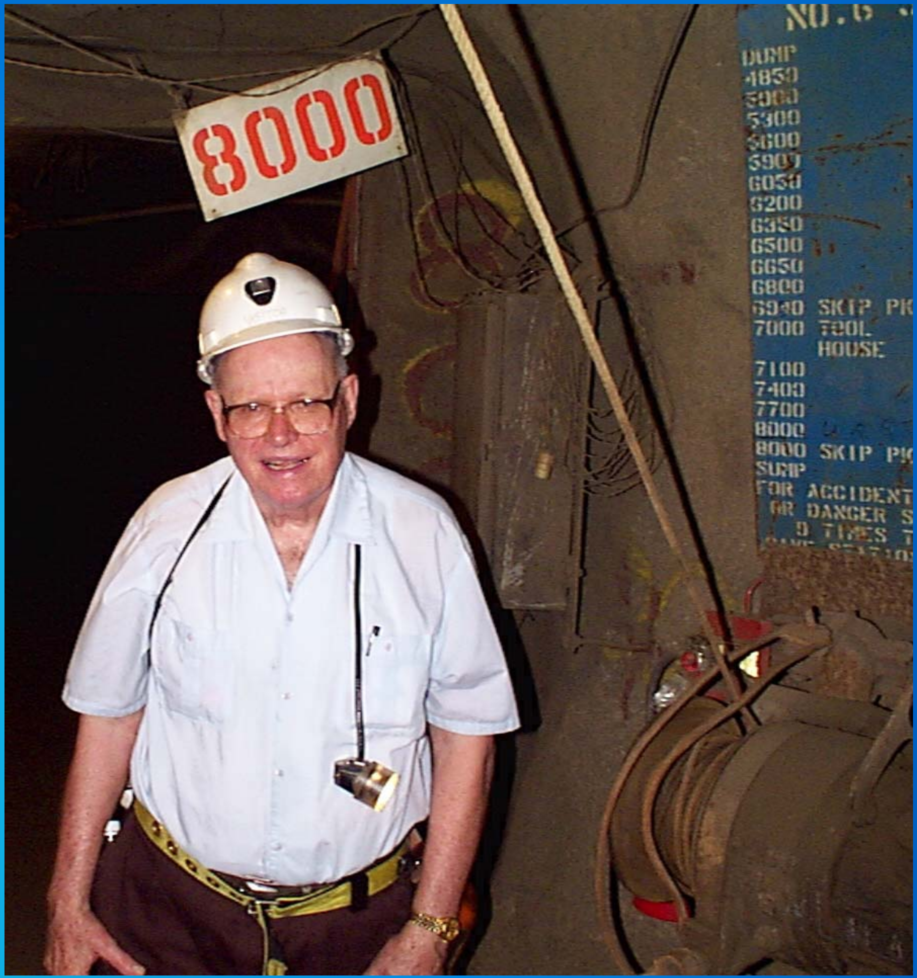
Homestake Mine 10/5/2001



Why use Homestake

- Deep access >8000 ft.
- Cheaper aseptic drilling of deep subsurface
- Multiple access points to follow geological and evolutionary history
- Better immediate access to sensitive samples (thermal)
- Areas that have been sequestered at different times over the last 120 years

Why Homestake? Cont.

- Enormous and very valuable data on geology and hydrology that can be integrated with biological assays
- Enormous possible test area for biogeochemistry and flow/transport studies
- Access to horizontal drilling in extraordinarily deep strata

Questions (native species)

- What are the dominant microbial communities in the deepest hard rock surfaces?
- Are the microbes in the deep strata unique?
- Have the microbes present contributed significantly to the geochemistry?
- What is the biomass or carbon mass balance?
- What are the critical environmental parameters that control community structure and activity?

Questions (native species) cont.

- Can the presence of life in fossilized hot springs be established from morphology and biogeochemistry
- Have changes in temperature, air, and water flow in the deep subsurface changed community structure and native geochemistry?

Questions (introduced species)

- How long do introduced species survive at different depths in the subsurface? (sources man, horses, air, water)
- Have introduced species displaced indigenous species?
- Have introduced species changed geochemical cycles?
- Have temperature, air, and water changes within the mine effected the survival of introduced species?
- What has been the natural attenuation rate of fuels, solvents, and PCBs at different depths?

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Benefits

- New Isolates that could facilitate bioremediation: heavy metals, radionuclides, hazardous organics
- Ability to determine activity in situ in the deep subsurface
- Ability to predict where certain types of biomineralization may have occurred and how
- Adaptions of life to extreme environments

Benefits 2

- Ability to model and predict flow and transport in the subsurface
- Understand flow and transport in the deep subsurface
- Determine the stability of injected carbon in the deep subsurface
- Determine critical parameters and feasibility of remediating the deep subsurface

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Benefits 3

- Establish chemical and morphological signatures for primitive life in fossilized hot springs
- Verify natural attenuation rates of contaminants in the deep subsurface
- Evolution of life on earth
- New Gene Pool!!!! The New Gold for Homestake!!!!